

1. A nucleic acid construct coding for a fusion polypeptide comprising a biologically active polypeptide that is a component of an intracellular signaling pathway, or a part thereof, and a GFP, with the proviso that the construct is not a construct coding for a fusion polypeptide in which the biologically active polypeptide is selected from the group consisting of PKC-alpha, PKC-gamma, and PKC-epsilon.

2. A nucleic acid construct coding for a fusion polypeptide comprising a biologically active polypeptide that is a component of an intracellular signaling pathway, or a part thereof, and an F64L mutant of GFP.

3. A nucleic acid construct according to claim 1 or 2, wherein the biologically active polypeptide is a protein kinase or a phosphatase.

4. A nucleic acid construct according to claim 1 wherein the GFP is N- or C terminally tagged, optionally via a peptide linker, to the biologically active polypeptide or part thereof.

5. A nucleic acid construct according to claim 1, wherein the biologically active polypeptide is a transcription factor or a part thereof which changes cellular localization upon activation.

6. A nucleic acid construct according to claim 1, wherein the biologically active polypeptide is a protein, or a part thereof, which is associated with the cytoskeletal network and which changes cellular localization upon activation.

7. A nucleic acid construct according to claim 1, wherein the biologically active polypeptide is a protein kinase or a part thereof which changes cellular localization upon activation.

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8. A nucleic acid construct according to claim 7, wherein the protein kinase is a serine/threonine protein kinase or a part thereof capable of changing intracellular localization upon activation.

9. A nucleic acid construct according to claim 7, wherein the protein kinase is a tyrosine protein kinase or a part thereof capable of changing intracellular localization upon activation.

10. A nucleic acid construct according to claim 7, wherein the protein kinase is a phospholipid-dependent serine/threonine protein kinase or a part thereof capable of changing intracellular localization upon activation.

11. A nucleic acid construct according to claim 7, wherein the protein kinase is a cAMPdependent protein kinase or a part thereof capable of changing cellular localization upon activation.

12. A nucleic acid construct according to claim 11, which codes for a PKAc-F64L-S65T-GFP fusion.

13. A nucleic acid construct according to claim 7, wherein the protein kinase is a cGMPdependent protein kinase or a part thereof capable of changing cellular localization upon activation.

14. A nucleic acid construct according to claim 7, wherein the protein kinase is a calmodulin-dependent serine/threonine protein kinase or a part thereof capable of changing cellular localization upon activation.

15. A nucleic acid construct according to claim 7, wherein the protein kinase is a mitogenactivated serine/threonine protein

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kinase or a part thereof capable of changing cellular localization upon activation.

16. A nucleic acid construct according to claim 15, which codes for an ERK1-F64L-S65TGFP fusion.

17. A nucleic acid construct according to claim 15, which codes for an EGFP-ERK1 fusion.

18. A nucleic acid construct according to claim 7, wherein the protein kinase is a cyclindependent serine/threonine protein kinase or a part thereof capable of changing cellular localization upon activation.

19. A nucleic acid construct according to claim 3, wherein the biologically active polypeptide is a protein phosphatase or a part thereof capable of changing cellular localization upon activation.

20. A nucleic acid construct according to claim 1 which is a DNA construct.

21. A nucleic acid construct according to claim 1 wherein the gene encoding GFP is derived from *Aequorea victoria*.

22. A nucleic acid construct according to claim 21 in which the gene encoding GFP is the gene encoding EGFP as defined herein.

23. A nucleic acid construct according to claim 21 in which the gene encoding a GFP is a gene encoding a GFP variant selected from F64L-GFP, F64L-Y66H-GFP and F64L-S65TGFP.

24. A DNA construct according to any one of claim 20, claim 22 or claim 23, which is a construct as identified by any of the DNA sequences shown in SEQ ID NO: 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, and 142, or is a variant thereof capable of encoding the

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same fusion polypeptide or a fusion polypeptide which is biologically equivalent thereto, as defined herein.

25. A cell containing a nucleic acid construct according to claims 1 and capable of expressing the sequence encoded by the construct.

26. A cell according to claim 25, which is a eukaryotic cell.

27. A cell according to claim 25, which is selected from the group consisting of fungal cells, such as yeast cells; invertebrate cells, including insect cells, and vertebrate cells, such as mammalian cells.

28. A cell according to claim 27, which is a mammalian cell.

29. An organism carrying in at least one of its component cells a nucleic acid sequence as contained in the constructs according to claim 1, said cell being capable of expressing said nucleic acid sequence.

30. An organism according to claim 29 which is selected from the group consisting of unicellular and multicellular organisms, such as a mammal.

31. A fluorescent probe comprising a GFP which is N- or C-terminally tagged, optionally via a peptide linker, to a biologically active polypeptide or a part or a subunit thereof which is a component of an intracellular signaling pathway as defined herein, the probe being a probe which is encoded by the nucleic acid construct according to claim 1.

32. An apparatus for measuring the distribution of fluorescence in at least one cell, and thereby any change in the distribution of fluorescence in at least one cell, which includes the following component parts: (a) a light source, (b) a means for

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selecting the wavelength(s) of light from the source which will excite the fluorescence of the protein, (c) a means for rapidly blocking or passing the excitation light into the rest of the system, (d) a series of optical elements for conveying the excitation light to the specimen, collecting the emitted fluorescence in a spatially resolved fashion, and forming an image from this fluorescence, (e) a bench or stand which holds the container of the cells being measured in a predetermined geometry with respect to the series of optical elements, (f) a detector to record the spatially resolved fluorescence in the form of an image, (g) a computer or electronic system and associated software to acquire and store the recorded images, and to compute the degree of redistribution from the recorded images.

33. An apparatus according to claim 32 in which some or all of the system is automated.

34. An apparatus according to claim 32 in which components (d) and (a) comprise a fluorescence microscope.

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35. An apparatus according to claim 32 in which component f is a CCD camera.

36. An apparatus according to claim 32 in which the image is formed and recorded by an optical scanning system.

37. An apparatus according to claim 32 in which a liquid addition system is used to add a known or unknown compound to any or all of the cells in the cell holder at a time determined in advance.

38. An apparatus according to claim 37 in which the liquid addition system is under the control of the computer or electronic system.

39. A method according to claim 1, wherein the method is a screening program for the identification of a biologically active substance as defined herein that directly or indirectly affects an intracellular signaling pathway and is potentially useful as a medicament, wherein the result of the individual measurement of each substance being screened which indicates its potential biological activity is based on measurement of the redistribution of spatially resolved luminescence in living cells and which undergoes a change in distribution upon activation of an intracellular signaling pathway.

40. A method of treating a condition or disease related to the intracellular function of a protein kinase comprising

administering to a patient suffering from said condition or disease an effective amount of a compound which has been discovered by any method according to the invention.

41. A compound that modulates a component of an intracellular pathway as defined herein, as determined by a method according to the method of the invention.

42. A medical composition comprising a therapeutic amount of a compound identified according the method of the invention.

43. A method of selectively treating a patient suffering from an ailment which responds to medical treatment comprising obtaining a primary cell or cells from said patient, transfecting the cell or cells with at least one DNA sequence encoding a fluorescent probe according to the invention, culturing the cell or cells under conditions permitting the expression of said probes and exposing it to an array of medicaments suspected of being capable of alleviating said ailment, then comparing changes in fluorescence patterns or redistribution patterns of the fluorescent probes in the intact living cell or cells to detect the cellular response to the specific medicaments (obtaining a cellular action profile), then selecting a medicament(s) based on desired activity and acceptable level of side effects and administering an effective amount of said medicament(s) to said patient.

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